의학석사 학위논문

High-Dose Chemotherapy and Autologous Stem Cell Rescue Therapy for High-Risk Neuroblastoma Patients

고위험군 신경모세포종 환자에서 고용량 항암화학요법과 자가 말초혈액 조혈모세포이식치료

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The Department of Molecular Oncology,

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High-Dose Chemotherapy and Autologous Stem Cell Rescue Therapy for High-Risk Neuroblastoma Patients

by
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A thesis submitted to the Department of Medicine in partial fulfillment of the requirements for the Degree of Master of Science in Molecular Oncology at Seoul National University College of Medicine

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Approved by Thesis Committee:

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논문 제목 : High-Dose Chemotherapy and Autologous Stem Cell Rescue Therapy for High-Risk Neuroblastoma Patients

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Abstract

Background: Neuroblastoma (NBL) accounts for 8% to 10% of all childhood cancers, and more than half of these cases are advanced stages. Vigorous research on single, tandem or triple high-dose chemotherapy (HDCT) and autologous stem cell rescue therapy have improved the survival of many high-risk NBL patients, however, patients remain to have a dismal prognosis.

In 2002, the Korean National Health Insurance policy extended coverage to include children over 1 year of age diagnosed with stage IV that showed partial response to surgery or chemotherapy and stage III in which complete resection was infeasible. As the burden of medical expenses was lifted, high-risk NBL patients were able to receive tandem HDCT more readily. The objective of this study was to conduct a historical analysis of patient outcome after the implementation of the Korean health care reform.

Methods: During the period between October 1997 and December 2010, a retrospective analysis was performed of 60 patients with stage III or IV NBL who received single or tandem HDCT with stem cell rescue therapy at Seoul National University Children's Hospital

(SNUCH). The overall survival (OS) and event-free survival (EFS) were analyzed.

Results: Sixty patients diagnosed with NBL received single or tandem HDCT and autologous stem cell rescue therapy. Five-year OS of the single and tandem group were 82.9% (95% CI, 69.4%-96.4%) and 85.6% (95% CI, 72.5%-98.7%), respectively (*P*=0.590). Five-year EFS of the single and tandem group were 52.9% (95% CI, 30.9%-67.4%) and 71.4% (95% CI, 43.3%-81.0%) (*P*=0.234). An event was more likely attributed to relapse, rather than treatment related mortality (TRM).

Conclusion: With the change of the Korean National Health Insurance policy, an improvement of outcome in NBL patients receiving autologous stem cell rescue was observed. However, relapse remains to be an issue. As subclinical disease activity is correlated with disease relapse, monitoring disease activity before relapse is crucial and identifying molecular targets for future therapies are warranted.

Key words: neuroblastoma, high-risk, tandem, high-dose chemotherapy, autologous peripheral blood stem cell transplantation

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LIST OF ABBREVIATIONS

ANC absolute neutrophil
ARF acute renal failure
COG Children's Oncology Group
EFS event-free survival
CI confidence interval
CCND1 cyclin D1
GD disialoganglioside
GABA gamma-aminobutyric acid
GABRB3 GABA A receptor, beta 3
G-CSF granulocyte colony-stimulating factor
HR hazard ratio
HDCT high-dose chemotherapy
ITT isotretinoin
ISL1 ISL LIM homeobox1
LMW low-molecular weight
KIF1A kinesin family member 1A
MEC Melphalan, Etoposide, Carboplatin
mMEC modified MEC
MRD minimal residual disease
NBL neuroblastoma
OS overall survival
PHOX2B paired-like homeobox 2b
PBSCM peripheral blood stem cell mobilization
PBSCT peripheral blood stem cell transplantation
PCR polymerase chain reaction
PFS progression-free survival
TMC Topotecan, Melphalan, Carboplatin
TRM treatment related mortality
TH tyrosine hydroxylase
VOD veno-occlusive disease
WBC white blood cell

Introduction

Neuroblastoma (NBL) is a neuroendocrine tumor, derived from neural crest tissues of the sympathetic nervous system. It accounts for 8% to 10% of all childhood cancers and is the most common extracranial solid tumor in childhood (1,2). As one of the most enigmatic disease entities exhibiting striking heterogeneity, it has been known to spontaneously regress when developed in those less than one year of age. Unfortunately, more than half of patients are advanced stages when diagnosed and continue to exhibit a dismal prognosis.

In 2002, the Korean National Health Insurance policy extended coverage to children over 1 year of age diagnosed with stage IV that showed partial response to surgery or chemotherapy and stage III in which complete resection was infeasible. Prior to March of 2002, NBL patients received myeloablative chemotherapy followed by a single course of autologous peripheral blood stem cell transplantation (PBSCT). However, after reforms in the Korean Health Care policy, patients were able to receive tandem HDCT with rapid bone marrow reconstitution as the burden of treatment expenses was lifted. This

change was in large part due to evidence of randomized pilot studies that showed that myeloablative consolidation combined with stem cell rescue therapy improved the dismal outcome of high-risk patients (3,4,5). However, treatment failure after high-dose chemotherapy (HDCT) followed by stem cell rescue therapy remains to be problematic with relapse, rather than treatment-related mortality (TRM), being the main cause (6).

Therefore, the objective of this study was to conduct a historical analysis of patient outcome after the implementation of the Korean health care reform. Patient characteristics and outcome of stage 3 or 4 NBL patients who received PBSCT at Seoul National University Children's Hospital (SNUCH) were reviewed. In addition, the causes of events in these patients were analyzed.

Patients and Methods

Patients

From October 1997 to December 2010, a retrospective analysis was performed of hospitalization records of 60 newly diagnosed NBL patients that received PBSCT at SNUCH of Seoul, Korea. With the change of National Health Care coverage in 2002, patients receiving autologous stem cell transplantation prior to March 2002 received single HDCT, whereas after March 2002, patients were able to receive tandem HDCT as the burden of medical expenses was lifted. The patients that received single HDCT are referred to as the single group, and those that received tandem HDCT are referred to as the tandem group. Patient sex, age, disease status, conditioning regimen received, infused cell number, and patient outcome were analyzed.

Induction and Consolidation

After diagnosis, patients received induction therapy that consisted of a minimum of 4-5 cycles of CCG-321-P2 (cisplatin, adriamycin, cyclophosphamide, vincristine) chemotherapy regimen (Table 1) (7). Patients were re-evaluated after induction and received surgical resection, if deemed a surgical candidate. After the completion of induction therapy, peripheral blood stem cell mobilization (PBSCM) was performed. These stem cells were cryopreserved and later infused back to the patient after the patient received myeloablative chemotherapy as autologous hematopoietic stem cell rescue therapy.

HDCT was defined as chemotherapy that induced bone marrow cell depletion to the state of necessitating stem cell rescue therapy. Patients that received single HDCT received either MEC (Melphalan, Etoposide, Carboplatin) or TMC (Topotecan, Melphalan, Carboplatin) regimen (8,9). Patients that received tandem HDCT received TTC (Topotecan, Thiotepa, Carboplatin), followed by MEC or mMEC (modified MEC) (Table 1).

In June of 2007, a modification of carboplatin dose was implemented to those receiving tandem HDCT, as patients were observed to experience renal toxicity. Patients that received 400mg/m^2 of carboplatin are referred to as the MEC group, and those that received 350mg/m^2 of carboplatin are referred to as the modified MEC (mMEC) group.

Post-HDCT or autologous SCT treatment consisted of 13-cis-retinoic acid (isotretinoin; ITT), IL-2, and radiotherapy. IL-2 was started when an increase of ANC greater than $1.0x10^9$/L and platelet count of greater than $50x10^9$/L without transfusion was observed after the completion of tandem HDCT. Local radiotherapy was initiated after day 28 of receiving stem cell rescue therapy and ITT was initiated after day 90.

Stem Cell Mobilization and CD 34+ Selection

Prior to receiving HDCT, all patients underwent the process of stem cell mobilization. After receiving peripheral blood stem cell mobilization (PBSCM) chemotherapy (Table 2), patients received subcutaneous injections of high dose G-CSF (10 μg/kg) starting from day 7 and until a white blood cell (WBC) count of $1.0x10^9$/L was achieved.

Patients received leukapheresis and $CD34^+$ cell selection was performed to decrease the potential of tumor contamination, after which the cells were cryopreserved. $CD34^+$ cell selection was performed using CliniMACS® CD34 Reagent System (Miltenyi Biotec, Bergisch Gladbach, Germany).

Supportive Care

As infection prophylaxis and supportive care plays a pivotal role in patient survival after SCT, we employed the following conservative management processes. Patients received low-molecular weight (LMW) heparin (Nadroparine) or lipo-PGE_1 (Eglandin) from the start of

HDCT as prophylaxis against veno-occlusive disease (VOD). Patients received granulocyte colony-stimulating factor (G-CSF) 300μg/m^2/d subcutaneously, starting on d1, and was discontinued when absolute neutrophil count (ANC) increased to more than 1.0x10^9/L for three consecutive days. If the patient spiked a fever of 38.0℃, broad spectrum antibiotics were administered. Patients received acyclovir, ciprofloxacin, micafungin, and isoniazid for prophylaxis against opportunistic infections.

Statistical Analysis

All data were analyzed using the standard statistical methods using the Statistical Package for the Social Sciences for Windows software (SPSS) version 19.0 program. Event-free survival (EFS) was defined as the time from diagnosis to the first event. An event was defined as occurrence of progression, relapse, or death. Overall survival (OS) was defined as the time from diagnosis to the time of death or time of last contact if the patient did not die. Survival data with 95%

confidence interval (CI) was obtained by performing Kaplan-Meier statistical analysis and log-rank test. Characteristics of the two groups were compared using Chi-square test or Fisher's exact test. Also, Cox-regression analysis was performed to evaluate the prognostic factors of variables at the time of diagnosis for EFS. *P*-value < 0.05 was determined to be statistically significant.

Table 1. Schema of induction and consolidation chemotherapy

Induction chemotherapy
Cisplatin 60mg/m^2 (day 1)
Etoposide 100 mg/m^2 (day 2)
Doxorubicin 30mg/m^2 (day 2)
Cyclophosphamide 30mg/kg (day 3,4)

Table 2. Schema of peripheral blood stem cell mobilization

PBSCM chemotherapy
Cyclophosphamide 1,000mg/m^2 (day 0 ~ day 2)
MESNA 1,500 mg/m^2 (day 0~ day 2)
Etoposide 150mg/m^2 (day 0 ~ day 2)
G-CSF 10 μg/kg (day 7~)

(PBSCM, peripheral blood stem cell mobilization; G-CSF, granulocyte colony-stimulating factor)

Table 3. Schema of high-dose chemotherapy prior to autologous stem cell rescue therapy

Single HDCT
MEC Melphalan 140mg/m^2 or 70mg/m^2 (day -7,-6)
Etoposide 200mg/m^2 (day -8 ~ -5)
Carboplatin 400mg/m^2 (day -8 ~ -5)
Or
TMC Topotecan 20mg/m^2 (day -8~-4)
Melphalan 140mg/m^2 or 70mg/m^2 (day -7 ~ -6)
Carboplatin 500mg/m^2 (day -5 ~ -3)
Tandem HDCT
TTC Topotecan 2mg/m^2 (day -8 ~ -4)
Thiotepa 300mg/m^2 (day -8 ~ -6)
Carboplatin 500mg/m^2 (day -5 ~ -3)
and
MEC Melphalan 140mg/m^2 or 70mg/m2 (day -7,-6)
Etoposide 200mg/m^2 (day -8 ~ -5)
Carboplatin 400mg/m^2 (day -8 ~ -5)
Or

mMEC	Melphalan 140mg/m^2 or 70mg/m2 (day -7,-6)
	Etoposide 200mg/m^2 (day -8 ~ -5)
	Carboplatin 350mg/m^2 (day -8 ~ -5)

(HDCT, high-dose chemotherapy; mMEC, modified MEC)

RESULTS

Characteristics of patients

As a retrospective analysis, this study observed the outcome of high-risk NBL patients treated with HDCT and autologous stem cell rescue therapy. Patient outcomes were investigated by both intention-to-treat and per-protocol analysis (10).

From October 1997 to December 2010, 60 newly diagnosed high-risk NBL patients received HDCT and stem cell rescue therapy at SNUCH of Seoul, Korea. Of the 60 patients newly diagnosed with high-risk NBL, 24 patients were designated to receive single HDCT, referred to as the single group and 36 patients were designated to receive tandem HDCT, referred to as the tandem group. Within the single group, one patient died from treatment-related cause of veno-occlusive disease (VOD), 11 patients relapsed, two patients could not be followed up, and 10 patients remain to be relapse-free. Of the 11 patients that relapsed, eight patients were lost in follow up, one patient died from

candidal infection, one patient is alive and receiving palliative chemotherapy, and one patient is alive and disease-free.

Out of the total of 36 patients who were designated to receive tandem HDCT, eight patients received single HDCT and 28 patients received tandem HDCT. Of the patients that received single HDCT, one patient died of acute renal failure (ARF) cause, three patients progressed, one patient relapsed, and three patients remain relapse-free. Of the 28 patients that received tandem HDCT, two patients died of TRM (specifically, ARF and septic shock), seven patients relapsed, and 19 patients remain relapse-free.

Therefore, 32 patients ultimately received single HDCT, and 28 patients received tandem HDCT. Patient outcome are summarized in Figure 1.

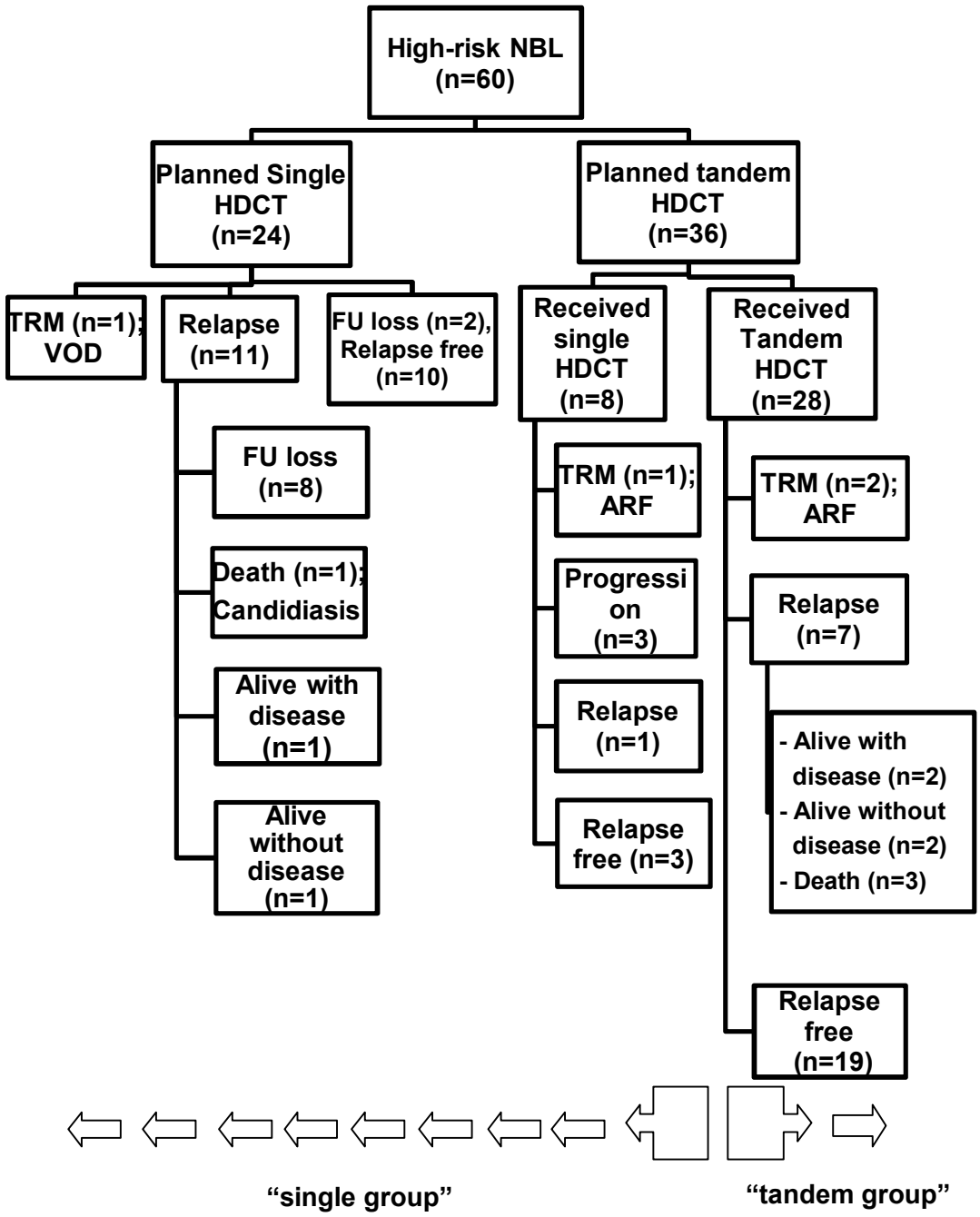


Figure 1. Patient Outcome

The median duration of follow up was 69.5 months (range, 10 - 198 months) for the single group and 64.6 months (range 9 – 137 months) for the tandem group. Patients ≥ 18 months of age consisted 78.1% (n=25) of the single group and 82.1% of the tandem group (n=23) (*P*=0.698). The primary site was most commonly located in the abdomen and MYCN amplification was present in 12.5% (n=4) of the single group and 60.7% (n=17) of the tandem group (*P* <0.001). Patients showed bone marrow metastasis at diagnosis in 78.1% (n=25) of the single group and 53.6% (n=15) of the tandem group. Bone metastasis at diagnosis was discovered in 84.4% (n=27) of the single group and 50.0% (n=14) of the tandem group. In order to reduce tumor cell contamination of PBSCs, positive selection of $CD34^+$ cells was performed in 62.0% (n=20) of cases in the single group and 92.9% (n=26) of the tandem group. The demographic data are summarized in Table 4.

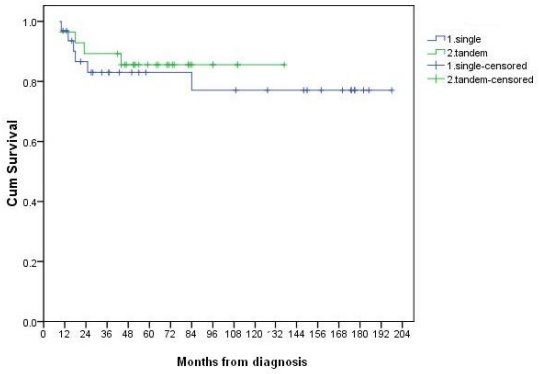
Table 4. Demographic data of patients.

Parameters	Single group (n=32)	Tandem group (n=28)	*P* value
Sex			
Female	25 (65.6%)	13 (46.4%)	0.342
Male	11 (34.4%)	15 (53.6%)	
Age at diagnosis			
< 18 months	7 (21.9%)	5 (17.9%)	0.698
≥ 18 months	25 (78.1%)	23 (82.11%)	
Primary site			
Abdomen	28 (87.5%)	22 (78.1%)	0.491
Extraabdominal	4 (12.5%)	6 (21.4%)	
N-myc amplification			
Amplified	4 (12.5%)	17 (60.7%)	
Not amplified	1 (3.1%)	7 (25.0%)	< 0.01
Not checked	27 (84.4%)	4 (14.3%)	
Bone marrow metastasis			
No	7 (21.9%)	13(46.4%)	0.058
Yes	25 (78.1%)	15 (53.6%)	
Bone metastasis			
No	5 (15.6%)	14 (50.0%)	0.004
Yes	27 (84.4%)	14 (50.0%)	
CD 34+ selection			
No	12 (37.5%)	0 (0%)	0.006
Yes	20 (62.0%)	28 (100.0%)	

Treatment Outcome

Patient outcome was analyzed per-protocol received. The 5-year OS and EFS of the single and tandem group were analyzed. Five-year OS of the single and tandem group were 82.9% (95% CI, 69.4%-96.4%) and 85.6% (95% CI, 72.5%-98.7%), respectively (*P*=0.590). Five-year EFS of the single and tandem group were 52.9% (95% CI, 30.9%-67.4%) and 71.4% (95% CI, 43.3%-81.0%) (*P*=0.234) (Figure 2). Multivariate analyses of the prognostic factors at diagnosis for EFS revealed bone metastasis at diagnosis to be significantly correlated with an unfavorable outcome (hazard ratio 2.82, 95% CI 1.06-7.05, *P*=0.0384) (Table 5).

(A)



(B)

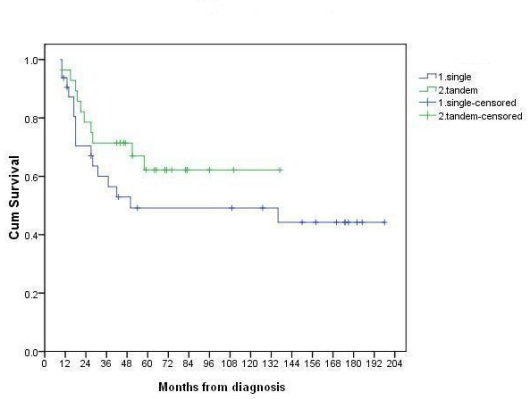


Figure 2. Kaplain-Meier analysis of patient outcome. Five-year overall survival (OS) and event-free survival (EFS) of the single and tandem group are depicted. (A) The OS of the single group was 82.9% (n=32) and 85.6% (n=28) in the tandem group (*P*=0.590). (B) Five-year EFS was 52.9% (n=32) in the single group and 71.4% (n=28) tandem group (*P*=0.234).

Table 5. Multivariate analysis of variables at diagnosis of EFS.

	Univariate analysis			Multivariate analysis		
Variables	HR	95% CI	*P* value	HR	95% CI	*P* value
Tandem HDCT	0.67	0.19-2.41	0.545	0.62	0.28-1.38	0.243
Male	1.03	0.29-0.64	0.968	1.44	0.64-3.23	0.381
Age at ≥ 18mon.	30.28	0.05->99	0.289	33.41	0.82->99	0.063
Abdominal tumor	0.48	0.12-1.86	0.289	0.24	0.08-1.45	0.146
MYCN Amplified	4.45	0.74-26.68	0.103	1.17	0.31-4.43	0.813
Bone marrow metastasis	0.84	0.24-2.99	0.713	1.66	0.70-3.96	0.252
Bone metastasis	1.29	0.33-5.00	0.713	2.82	1.06-7.50	0.038
$CD34^+$ selection	1.11	0.23-5.22	0.898	1.09	0.43-0.80	0.850

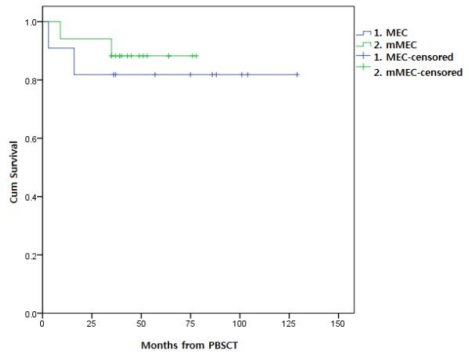
(HR, hazard ratio; CI, confidence interval)

The major cause of events in patients was observed to be relapse, as opposed to TRM. Twelve of 32 patients relapsed in the single group and TRM attributed to VOD and ARF was noted. Seven of 28 patients relapsed in the tandem group and 2 cases of TRM related to ARF were noted.

As patients were observed to experience renal toxicity, a decrease in carboplatin dosage from 400mg/m^2 to 350mg/m^2 was implemented in the second myeloablative conditioning regimen in June of 2007. Of the patients that received tandem HDCT, patients that received 400mg/m^2 of carboplatin are referred to as the MEC group and those that received a modified dose 350mg/m^2 of carboplatin are referred to as the mMEC group. Five-year OS and EFS of the two groups were compared and are depicted in figure 3. In comparing the MEC and mMEC group, EFS was defined as the time from PBSCT to the first event and OS was defined as the time from PBSCT to the time of death or last time of follow up if the patient did not die. The 5-year OS in the MEC and mMEC group were 81.8% (n=11) and 88.2% (n=17),

respectively (*P*=0.611). The 5-year EFS of the MEC and mMEC group were 54.5% (n=11) and 70.6% (n=17), respectively (*P*=0.400).

(A)



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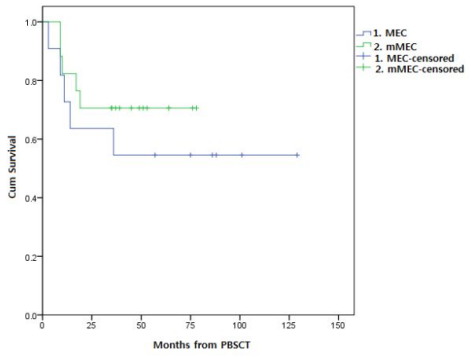

Figure 3. Of the patients that received tandem HDCT, survival of patients that received different doses of carboplatin during myeloablative conditioning. The MEC group received 400mg/m^2 of carboplatin and mMEC group received 350mg/m^2 of carboplatin. (A) Five-year OS of the MEC and mMEC group were 81.8% (n=11) and 88.2% (n=17), respectively (*P*=0.611). (B) Five-year EFS of the MEC and mMEC group were 54.5% (n=11) and 70.6% (n=17), respectively (*P*=0.400).

DISCUSSION

As tandem HDCT has shown to improve the survival of patients with high-risk NBL, it was included in the National Health Insurance coverage in the year 2002. The objective of this study was to investigate the clinical characteristics and outcome of patients that received single and tandem HDCT combined with autologous stem cell rescue therapy as a result of health care reform. Also, investigation into the events that caused treatment failure was initiated.

In 2010, the Korean Society of Pediatric Hematology-Oncology reported of the 5-year EFS of patients that received tandem HDCT and stem cell rescue therapy. The results showed higher survival in patients that received tandem HDCT with 5-year EFS of 51.2 ±12.4% (*P*=0.030) (11). In the present study, the 5-year OS and EFS of the single and tandem groups were obtained. The outcome of patients that received tandem HDCT suggests that an improvement of OS and EFS in patients with high-risk NBL is plausible. The Children's Oncology

Group (COG) has previously reported of a cooperative group setting for tandem autologous PBSCT with overall 5-year progression-free survival (PFS) and OS of 24.2% ± 7.5% and 36.4% ± 8.4% (n=33) (12).

The main cause of an event in patients appeared to be relapse. Although the results of 5-year OS and EFS appeared promising, investigation into the 10-year OS and EFS of the two groups revealed that relapse was observed after 5 years of receiving stem cell rescue therapy in the single group. The 10-year OS of the single and tandem groups were 77.1% (95% CI, 60.2%-93.9%) and 85.6% (95% CI, 72.4%-98.7%) (*P*=0.590). This suggests that a long-term follow up period is needed. Other studies have also supported this finding that relapse or tumor progression rather than TRM is the cause of treatment failure (13). In one study reported by George et al., the majority of relapses occurred at metastatic sites, most commonly at the bone, bone marrow, and liver (14).

In the present study, a greater incidence of relapse was observed

in the single group, and 4 of the 8 patients that were intended to receive tandem HDCT but received single HDCT for other causes was due to disease progression or relapse. This leads to the thought that a shorter interval between the first and second HDCT may decrease relapse rates and warrants further investigation. A study of rapid sequence tandem HDCT was explored by Grupp et al. and yielded a 3-year EFS of 63% (15). In one report, Philips et al. (16) reported of using tandem HDCT with bone marrow as the source of stem cells. The regimen required a significant time interval between stem cell rescue and TRM was reported from delayed engraftment (17). Therefore, an optimal interval between PBSCTs has yet to be defined.

As targeting subclinical disease activity is correlated with disease relapse, efforts of monitoring disease activity before relapse have been actively pursued. Accurate monitoring of minimal residual disease (MRD) with molecular markers could sophisticate the optimal timing of surgery and tandem HDCT for a specific patient. Polymerase chain reaction (PCR) detection of MRD has been used to monitor therapy response with markers, such as tyrosine hydroxylase (TH) and

disialoganglioside (GD)2 synthase, however their uses are limited in that they are also expressed in normal peripheral nerves and the cerebellum (17). Nonetheless, successful treatment with anti-GD2 monoclonal antibody to prevent relapse has been reported (18). Methods of measuring MRD with novel markers have been proposed and include cyclin D1 (CCND1), a cell-cycle control gene (19), gamma-aminobutyric acid (GABA) A receptor, beta 3 (*GABRB3*), ISL LIM homeobox1 (ISL1), kinesin family member 1A (KIF1A) and PHOX2B (17) (paired-like homeobox 2b). These molecular markers were found to be significantly accurate in testing the progress-free survival (PFS) of NBL patients (20).

In the present study, TRM was observed to be attributed to renal toxicity and a lower incidence was observed in patients who received lower doses of carboplatin. Therefore, it was hypothesized that a conditioning regimen related to renal toxicity may play a factor in increasing TRM. After making adjustment to the dose of carboplatin, patient outcome suggests that an improvement in OS and EFS could be achieved (P=0.611, P=0.400). Although the EFS and OS were not

statistically significant between the single and tandem group, improved OS and EFS in high-risk NBL patients was suggested, which may also be due to the improved salvage therapy and supportive care. In other words, it is important to note that an improved outcome after myeloablative chemotherapy followed by stem cell rescue therapy should be viewed in context of the complete therapy, with consideration of the salvage therapy and previous therapy of induction chemotherapy, surgery, and/or radiotherapy (1).

The issue of $CD34^+$ selection in autologous SCT has been controversial, as definitive evidence of its benefits have been unclear. Recent evidence suggests that CD34 expression does not necessarily correlate with stem cell activity (21). In concept, the process of positively selecting CD34 cells and purging PBSCs is performed to reduce tumor contamination and has been shown to improve outcome for patients with high-risk NBL (22). However, a randomized phase 3 trial conducted by the COG (COG A3973) has established that *ex vivo* purging of PBSCs for autologous PBSCT did not improve outcome of high-risk NBL patients (1). In the present study, definitive evidence of

the benefits of $CD34^+$ selection could not be delineated and warrants further investigation with a larger pool.

Limitations of this study include the retrospective nature of the analysis and a lack of a long-term follow up period. Also, because patients were not allocated single or tandem HDCT during the same time period, it is difficult to conclude that the improvement of patient outcome is solely due to the effects of tandem HDCT. As the improvement of other salvage therapies that accompanied the passage of time may confound benefits of tandem HDCT.

Also, it should be noted that eight of the 11 patients that relapsed in the single group were lost in follow up. Their data were censored off during analysis, and censoring also affects survival rates. Because censoring removes subjects from the dominator, such as individuals still at risk, a large number of patients lost in follow up influences the survival rates of the group (23).

In conclusion, the outcome of high-risk NBL patients has improved with the implementation of the Korean National Health Insurance policy

reform. As relapse is the main cause of treatment failure for NBL patients receiving PBSCT, future trials should be directed on monitoring disease activity from the molecular level and evaluating the effectiveness of a shorter time interval between PBSCTs. As patient outcome differs from each institution, future trials with carefully designed regimens that investigate the optimal induction and myeloablative regimen with long term follow-up periods are warranted.

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